Genetic Polymorphism of Thiopurine Methyltransferase and Thiopurine Therapy

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Genetic polymorphism of thiopurine methyltransferase and thiopurine therapy
From molecular genetics to clinical medicine

• Azathioprine, mercaptopurine, thioguanine
• Inactivated by a polymorphic enzyme
• Toxicity determined by TPMT genotype

Mercaptopurine metabolism in hematopoietic tissues

Evans et al., SJCRH, 2000

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Azathioprine metabolism

\[
\begin{align*}
\text{AZA} & \rightarrow \text{MP} \rightarrow \text{HPRT} \rightarrow \text{TGN} \\
& \downarrow \text{TPMT} \downarrow \text{XO} \\
& \downarrow \text{MeMP} \downarrow 6\text{Tu} \\
& \text{(inactive)}
\end{align*}
\]

TPMT methylates (inactivates) 6MP

Contribution of genetic polymorphisms to drug metabolism

Evans WE and Relling MV, Science 286:487-91, 1999

Proportions based on estimated number of drug substrates
How does MP exert its antileukemic effects?

Incorporation into DNA:
- Inhibits DNA replication (e.g., RNaseH, TopoII)
- Triggers apoptosis via:
  - MSH2 dependent mechanism (p53 dependent)
  - HMGB1/GAPDH/Hsp70/Erp60

Inhibition of DNPS:
- Via MeMPR

How 6MP kills leukemia cells: TG incorporation into DNA
Local structural modifications of duplex DNA by thioguanine incorporation (NMR Structure, JBC 2003)

Thioguanine in DNA alters the DNA repair enzyme topoisomerase II function

ThioG substitution effects on topo II cleavage

Somerville et al., JBC, 2003

Somerville et al., JBC, 2003

FASEB J 14:2339-44, 2000

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Thioguanine incorporation in DNA alters RNaseH function

<table>
<thead>
<tr>
<th>Duplex*</th>
<th>Structure</th>
<th>Relative RNase H cleavage (%)</th>
<th>Nuclear extract</th>
<th>X-ids</th>
<th>Y-ids</th>
</tr>
</thead>
<tbody>
<tr>
<td>V (X=dG)</td>
<td>5'-UAGCGCGGUGCUGGUCCACCAU-3'</td>
<td>Eco Col RNase H 150 76 ± 2</td>
<td>Molt</td>
<td>72 ± 4</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>VI (X=dG)</td>
<td>3'-FGCGCAACA-5'</td>
<td>CEM</td>
<td>58 ± 2</td>
<td>6 ± 1</td>
<td></td>
</tr>
<tr>
<td>VII (5'=dG)</td>
<td>5'-UAGCGCGGUGCUGGUCCACCAU-3'</td>
<td>E.Coli RNase H</td>
<td>55 ± 1</td>
<td>15 ± 2</td>
<td></td>
</tr>
<tr>
<td>VIII (3'=dG)</td>
<td>3'-FGCGCAACA-5'</td>
<td>E.Coli</td>
<td>69 ± 1</td>
<td>19 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

* In all experiments duplex concentration C0 = 0.2x10^-6M

Krynetskaia et al., Mol Pharm, 1999

Role of Postreplicative DNA Mismatch Repair in the Cytotoxic Action of Thioguanine
Peter F. Swan,* Timothy P. Waters, David C. Moulton, Yiao-Zhong Xu, Qingguo Zheng, Min Edwards, Raymond Mace

It is proposed here that the delayed cytotoxicity of thioguanine involves the postreplicative DNA mismatch repair system. After incorporation into DNA, the thioguanine is chemically modified by f-adenylylation to form S'-methylythioformycin. During DNA replication, the S'-methylythioformycin directs incorporation of either thymines or adenosines into the growing DNA strand, and the resultant S'-methylthioformycin-thymine pairs are recognized by the postreplicative mismatch repair system. Apoptosis, an immunoreceptor used in organ transplantation, is partly concerted to thioguanine. Because the carcinogenicity of S'-methylythioformycin depends on formation of S'-methylythioformycin in DNA, the formation of the analog S'-methylythioformycin may partly explain the high incidence of cancer after transplantation.

Science August 1996, 273

MSH2 facilitates activation of Chk2

Alternative mechanism to trigger apoptosis in MSH2- ALL

Krynetski et al., Ca Res 2003
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MeTIMP inhibits DNPS

Inhibition of DNPS in ALL cells after *in vivo* MP treatment

Antileukemic effects correlated with inhibition of DNPS

- No inhibition
- Partial inhibition
- Full inhibition

Change in leukocyte counts from day 0 to day 3

-40%
-30%
-20%
-10%
0%

Note: 6MP alone had much lower effect on DNPS than MTX or MTX+MP

Multiple mechanisms by which MP exerts antileukemic effects

Incorporation into DNA:
Inhibits DNA replication (*e.g.*, RNaseH, TopoII)

Triggers apoptosis via MSH2 dependent mechanism (*p53* dependent)

**HMGB1/GAPDH/Hsp70/Erp60**

Inhibition of DNPS: via MeMPR
Why is TPMT genetic polymorphism important for MP?

Mercaptopurine metabolism in hematopoietic tissues

Evans et al., SJCRH, 2000

Lenard et al., Lancet, 336:225-9, 1990

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Human RBC TMPT

TPMT genetic polymorphism determines mercaptopurine (6MP) metabolism
Our cancer prototype

Inheritance of TPMT activity
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Pulse-chase analysis of rh TPMT in COS-1 cells

*1

*2

*3A

*3B

*3C

T_{1/2A} = 0.35 h (T_{1/2B} = 41 h)

T_{1/2B} = 0.67 h

T_{1/2B} = 5.5 h

T_{1/2B} = 11.2 h


TPMT Deficient Heterozygous Homozygous

TPMT protein (density of western blot/2×10^6 RBC)

Erythrocyte TPMT activity (U/mL pRBC)

PCR based genotyping method

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Human TPMT gene and mutant alleles

>95% concordance between TPMT genotype and phenotype

Disclosure: US Patent (1995) for molecular diagnostics (genotyping) based on these 3 TPMT SNPs and alleles.
Sensitivity = 90%; Specificity = 99%
Schaefeler et al., PGEN, 2004 (n=1214)

Thiopurine S-methyltransferase (TPMT) activity in patients with different TPMT genotypes determined by mutation – specific polymerase chain reaction methods


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Estimated TPMT allelic frequencies for the African-American and Caucasian populations

<table>
<thead>
<tr>
<th>Allele</th>
<th>Estimated allelic frequency in population (% [95% CI])</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMT*2</td>
<td>0.4 [0.4-0.5]</td>
<td>NS</td>
</tr>
<tr>
<td>TPMT*3A</td>
<td>0.8 [0.6-0.9]</td>
<td>0.007</td>
</tr>
<tr>
<td>TPMT*3C</td>
<td>0.2 [0.1-0.4]</td>
<td></td>
</tr>
<tr>
<td>TPMT*6</td>
<td>0.08 [0.05-0.1]</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.17 [0.13-0.2]</td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>90.4</td>
<td>96.3</td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hon et al., Human Molecular Genetics 8(2):371-6, 1999

TPMT variant alleles in various world populations

TPMT non-functional mutant alleles

<table>
<thead>
<tr>
<th>Allele</th>
<th>% of mutant alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMT*2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G238C (Ala→Pro)</td>
</tr>
<tr>
<td>TPMT*3A</td>
<td>17%</td>
</tr>
<tr>
<td></td>
<td>G640A (Ala→Thr)</td>
</tr>
<tr>
<td></td>
<td>A719G (Tyr→Cys)</td>
</tr>
<tr>
<td>TPMT*3C</td>
<td>52%</td>
</tr>
<tr>
<td></td>
<td>GT19G (Tyr→Cys)</td>
</tr>
</tbody>
</table>

(1) S.E USA, (2) Ghanaians, Kenyan, (3) Japanese, Chinese (4) US, UK, France

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The importance of TPMT haplotype for common SNPs

Very rare TPMT alleles

Molecular haplotyping of genomic DNA for multiple single-nucleotide polymorphisms located kilobases apart using long-range polymerase chain reaction and intramolecular ligation

Oliver G. McDonald, Eugene Y. Krzywinski and William E. Evans

Pharmacogenetics 2002, 12:1-7
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Drug exposure determined by TPMT genotype

**Krynetski and Evans, Pharm Res 16(3):342-9, 1999**

Hematological toxicity determined by TPMT genotype

**Relling et al., JNCI, 1999 years**

TPMT genotype and tolerance of azathioprine therapy

**Black, McLeod, Capell et al., Ann Intern Med 1998**

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TMPT genotype in thiopurine intolerant patients


Thiopurine methyltransferase (TMPT) genetic polymorphism and 6MP dose requirement

Evans et al., SJCRH, 2000

6MP dosage reduction in TPMT deficient all patient

MCAD/MMP (PO)

TPMT deficient (<1) n=1
NL TPMT (7.5-37) n=30

RBC 6TGN

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Fatal toxicity from azathioprine in a TPMT-deficient patient

Schuetz et al., Lancet 341:436, 1993

Dosing 6MP without pharmacogenetics

Cheok and Evans, Nat Rev Cancer 6:117, 2006

NB: 6MP dose reduction based on TPMT genotype did NOT influence ALL relapse

Relling et al., Blood, 2006
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TPMT-KO mouse recapitulates human TPMT phenotypes

Hartford, Ca Res 2007

Interactions of genetics polymorphisms and treatment may result in adverse effects

Evans et al., SJCRH, 2000

TPMT deficiency associated with higher risk of radiation-Induced brain tumors in patients receiving 6MP + CNS radiation

Estimated cumulative incidence of radiation-associated secondary malignant brain tumor for seven children in total 30 who received preventive cranial radiotherapy and had genetic defects in thiopurine methyltransferase compared with 45 with wild-type status

Relling et al., Lancet, 354:34-39, 1999
Thioguanine in DNA alters the DNA repair enzyme topoisomerase II function

**ThioG substitution effects on topo II cleavage**

- Topo II
- Topo II + Etoposide

**Figure:**

FASEB J 14:2339-44, 2000

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**TPMT and ITPA**

(inosine triphosphatase) exhibit genetic polymorphism (SNPs)

- Methyl-mercaptopurine
- Methyl-thio-IMP
- Methyl-thio-GMP

**Figure:**

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**ITPA genotype and median concentrations of 6MP metabolites in RBC during continuation treatment with MP**

- [wt TPMT] (Total XIIIIB)

**Figure:**

(G. Stocco et al., 2008)
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Effects of ITPA when TPMT is non-functional but MP dose adjusted for TPMT genotype?

ITPA & TPMT genotypes and concentrations of mercaptopurine metabolites in RBC during continuation phase treatment with MP (Total XIIIIB)

6MP induced toxicity (fever and neutropenia)
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Potential of pharmacogenomics

1. Genetic profile for non-response or toxicity
   - Treat with alternative drug or dose

2. Genetic profile for favorable response
   - Treat with conventional drug or dose

Evans WE and Relling MV, Science 286:487-91, 1999

Evans et al., SJCRH, 2000

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